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Comparison of PCR-based molecular marker analyses of *Musa* breeding populations

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Abstract

Progress in the breeding of plantain and banana has been restricted by the complex genetic structure and behaviour of cultivated polyploid *Musa*. Genetic improvement has been hindered due to the large amount of space required for growth and maintenance of plant populations, in addition to the long growth cycle and the low levels of fertility and seed viability characteristic of cultivated genotypes. Molecular marker assisted breeding has the potential to dramatically enhance the pace and efficiency of genetic improvement in *Musa*. This study was conducted to compare different PCR-based marker systems (RAPD, VNTR and AFLP) for the analysis of breeding populations generated from two diverse *Musa* breeding schemes. All three assays detected a high level of polymorphism between parental genotypes and within progeny populations. As expected, AFLP assays had by far the highest multiplex ratio while VNTR analysis detected the highest levels of polymorphism. AFLP analysis of a full-sib tetraploid hybrid population confirmed previous reports based on VNTR analysis, of a high frequency of recombination during $2n$ $(3x)$ gamete formation by a triploid plantain landrace. In addition, both VNTR and RAPD analyses of a full-sib triploid hybrid population suggested a high frequency of homoeologous recombination during n $(2x)$ gamete formation by tetraploid hybrids. In general, there was a poor correlation between estimates of genetic similarity based on different types of marker. The implications of these findings for the molecular breeding of *Musa* crops are discussed.

Abbreviations: AFLP, amplified fragment length polymorphism; IITA, International Institute of Tropical Agriculture; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat; TMPx, tropical *Musa* plantain-derived tetraploid hybrid; USDA, United States Department of Agriculture; VNTR, variable number of tandem repeats.

Introduction

Plantain and banana are triploid ($2n = 3x = 33$) giant perennial herbs of considerable importance to the agriculture of tropical humid forest regions in Africa, Central and South America, and Asia [8, 42]. Increases in *Musa* productivity have traditionally relied on improvements in crop husbandry. However, considerable advances have recently been made in understanding the genetic basis of important agronomic characters in these crops [27, 63]. Furthermore,

promising tetraploid hybrids have been generated by several breeding programs across the world [6, 43, 61, 62]. Although these hybrids produce high-yielding bunches in comparison to landraces, they exhibit only a fraction of the yield potential of this crop [57].

Musa breeding is largely dependent on ploidy manipulations through interspecific and interploidy hybridizations [29] (Figure 1). The genetic behaviour during such crosses and the genetic structure of the resultant allopolyploid hybrids appears to be highly complex [4, 31]. *Musa* breeding is time consuming and expensive due to the large amount of space required for the cultivation of these crops (6 m^2 per plant) and their long growth cycles (10 to 18 months). The combination of these factors suggests that the pace and efficiency of plantain and banana improvement could be greatly enhanced through molecular marker-assisted breeding.

Molecular markers based on PCR are the most appropriate assays for molecular breeding applications due to their relatively simple protocols and ease of automation [39]. Random amplified polymorphic DNA (RAPD) analysis has been particularly popular as it requires no prior knowledge of the genome. Analysis of the variable number of tandem repeats (VNTR) of microsatellites has been reported to detect abundant polymorphism in many systems [36], including *Musa* [4, 17]. More recently, amplified fragment length polymorphism assays (AFLP) [65] have been demonstrated to have a very high multiplex ratio (average number of alleles detected per assay) in a number of systems including potato [54], rice [1] and soybean [18]. AFLP analysis also has the advantage of not requiring prior knowledge of the genome.

Reports of the application of molecular markers in *Musa* have primarily concentrated on the analysis of diverse germplasm (reviewed in [5]). Comparison between restriction fragment length polymorphisms (RFLP) and VNTR analysis in *Musa* has been reported [17]. However, RFLP analysis may not detect sufficient polymorphism between closely related genotypes [13, 20] and it is not readily amenable to the high throughput demands of molecular breeding applications [39]. Previous studies comparing various PCR-based DNA marker assays have tended to concentrate on diverse germplasm within a single species [21, 23, 25, 37, 45, 47]. However, it is expected that the comparative advantage of different DNA assays will vary with crop and species specific qualities such as genome size and structure, and extent of detectable polymorphisms. In addition, various DNA marker assays are known to have differential comparative advantages when applied in different breeding systems and species. Thus, it is important to study the extent to which phenomena observed in such reports are relevant to the analysis of progeny populations from interspecific and interploidy crosses which form the basis of *Musa* breeding programmes across the world. For these reasons, this study was conducted to compare three major PCR-based marker systems (RAPD, VNTR and AFLP) for use in molecular breeding of *Musa*.

Materials and methods

Plant material

Tetraploid hybrids

A total of 990 bagged inflorescences of the triploid plantain landrace, Obino l'Ewai, were crossed with the diploid wild banana, *Musa acuminata* subsp. *burmannica* clone Calcutta 4 (hereafter Calcutta 4). These crosses generated 4871 seeds, from which 16 tetraploid hybrids were recovered of which 14 were selected due to their resistance to the black sigatoka disease [60]. The ploidy level of these hybrids was confirmed through morphological evaluation and chromosome counts [53]. Field-grown plants (IITA Onne Station, Nigeria) of hybrid and parental genotypes were used for isolation of DNA (Onne Station) as described previously [4]. Part of each DNA sample was then used for microsatellite marker analysis at Onne Station or AFLP analysis at the University of Gent. The following 14 black sigatoka resistant hybrids were used: TMPx 548-4, TMPx 548-9, TMPx 2637-49, TMPx 4698-1, TMPx 4744-1, TMPx 5511-2, TMPx 5706-1, hybrid 5860-1, TMPx 6930-1, TMPx 7002-1, TMPx 7356-1, hybrid 11669-1, hybrid 14563-1 and hybrid 15063-1.

Putative triploid hybrids

A single bagged inflorescence of the tetraploid hybrid TMPx 4698-1 was backcrossed to the paternal genotype, Calcutta 4. This cross generated 420 seeds of which 134 contained embryos from which 31 seedlings were recovered through *in vitro* embryo germination as described previously [58]. A total of 28 individuals (hereafter referred to as BC1 to BC28) survived nursery hardening and field establishment. Field-grown plants (IITA Onne Station, Nigeria) of backcross individuals, parental and grandparental

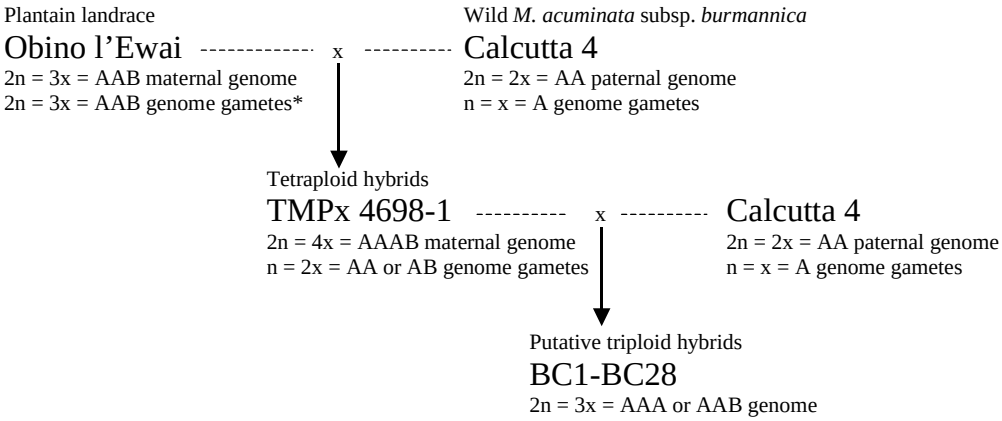

Figure 1. Diagrammatic representation of the pedigrees for breeding populations analyzed in this study. (*Obrino l'Ewai also produces n = x gametes leading to the generation of diploid hybrids.

genotypes were used for isolation of DNA for microsatellite and RAPD analyses at Onne Station as described previously [4].

PCR-based assays

RAPD analysis

Random decamer primers A1-A19 and B1-B19 were obtained from Operon (Alameda, USA). The PCR reactions each contained 5–10 ng template DNA, 2.5 mM Mg^{2+}, 10 mM Tris-HCl pH 9, 0.2 mM of each dNTP, 1 unit *Taq* DNA polymerase (Red Hot, Advanced Biotechnologies, UK), 0.6 μM primer in a reaction volume of 15 μl. Templates were initially denatured for 4 min at 94 °C followed by 40 amplification cycles each consisting of 50 s denaturation at 94 °C, 50 s annealing at 35 °C and 90 s extension at 72 °C. Amplifications were carried out in a Perkin Elmer model 9600 thermal cycler. PCR products were separated electrophoretically on 1.5% agarose gels (Appligene or Sigma) containing 0.3 μg/ml ethidium bromide, in 1× TBE buffer at 5 V/cm. RAPD bands were visualized and photographed using UV illumination. RAPD assays generating weak or ambiguous amplification products were repeated up to three times to confirm the consistency of these markers. Amplification product profiles were scored for the presence or absence of bands.

Microsatellite analysis

A genomic library was generated from DNA isolated from *M. acuminata* subsp. *malaccensis* [14]. Clones from this library which contained microsatellites were sequenced and primers designed from flanking regions (at USDA) as described previously [4], to generate microsatellite markers with Ma prefixes. Primers were designed using Primer Design software (Research Genetics, USA). Primer sequences for additional microsatellite markers (prefix STMS and prefix CIR) were generated independently as described elsewhere [17]. All primers were synthesized by MWG-Biotech (Germany).

PCR reactions each contained 25 ng template DNA, 1.2 μM each of forward and reverse primer, 10 mM Tris-HCl pH 9, 2.5 mM Mg^{2+}, 0.2 mM each dNTP and 1 unit *Taq* polymerase (Appligene) in a reaction volume of 15 μl. Reactions were initially denatured for 4 min at 94 °C followed by 30 amplification cycles each consisting of 1 min. denaturation at 94 °C, 1 min annealing at primer melting temperature (specific for each primer, see Table 1 and in [4, 17]), 45 s extension at 72 °C. Amplifications were carried out in a Perkin Elmer model 9600 thermal cycler. PCR products were separated electrophoretically on 1.5% w/v Nusieve GTG (FMC) + 1.5% w/v Metaphor (FMC) + 0.5% Multipurpose (Appligene) agarose gels containing 0.3 μg/ml ethidium bromide, in 1× TBE buffer at 5 V/cm for ca. 4 h. VNTR bands were visualized and photographed using UV illumination. Amplification product profiles were scored for the presence or absence of bands as generally allelic relationships could not be defined due to the high multiplex ratio of most assays.

The following primers generated microsatellite amplification products when prescreening the parental genotypes: Ma 0-9, Ma 1-2, Ma 1-16, Ma 1-17, Ma 1-19, Ma 1-24, Ma 1-27, Ma 1-29, Ma 2-4, Ma 2-4B, Ma 2-7, Ma 2-10, Ma 3-1N, Ma 3-41, Ma 3-46,

Table 1. Sequence and annealing temperatures of previously unpublished primers for *Musa* microsatellites generating polymorphic amplification products in this study.

Clone	Primer sequence (5′ to 3′)	Annealing temp. (°C)
Ma 0-9	ACGGTGATGAAAGCTTACACG GTGGCCGAAAACACAACC	57
Ma 1-19	ATTGGGCAGGCATCAAGTAC GCAATGGTGCTACCCACC	60
Ma 1-29	AGTCACGGAGCATATTTGGG TACTCAAGCTATGCATCCAACG	57
Ma 2-4B	TCACGAAACACTGAAAAGCG TTTTCCTCCCCGGAAAAG	60
Ma 2-10	GGGTTCCGTGAAGATTGATT TGGACAACTGACGACCATAAT	60
Ma 3-1N	ACGATCTGGCTGAGAATTGG TCTCTATGGATTGAAACCACCC	60
Ma 3-64	CAACAGCTCTCGCACTTC AACCTTTAATGTTGGATCTGC	58
Ma 3-132	AACGCGAATGTGTGTTTTCA TCCCTCTTCAACCAAAGCAC	60
Ma 3-161	AAACGTGAAACGACAGCTTCTG TCCGGCTTCGAATTGAATG	62

Ma 3-48, Ma 3-50, Ma 3-55, Ma 3-59, Ma 3-60, Ma 3-64, Ma 3-77, Ma 3-79, Ma 3-81, Ma 3-90, Ma 3-92, Ma 3-103, Ma 3-104, Ma 3-109, Ma 3-127, Ma 3-132, Ma 3-139, Ma 3-161, CIR 37, CIR 38a, CIR 38b, CIR 276, CIR 1113, CIR 332a, CIR 327a, CIR 327b, CIR 631a, STMS 7, STMS 8, STMS 9, STMS 12, STMS 14, STMS 15. The sequences of forward and reverse primers with STMS and CIR prefixes have been reported elsewhere [17]. The sequences of primer pairs with Ma prefixes (excluding those described previously [4]) are listed in Table 1. These microsatellite markers cover a wide range of core motifs including perfect di- and trinucleotide repeats, compound dinucleotide repeats plus imperfect di- and trinucleotide repeats (comprising GA, AT, CT, GAA, CTT or ATT) as described previously [3].

AFLP analysis

The protocol for AFLP analysis of *Musa* accessions was adapted at the University of Gent from that reported by Vos *et al.* [56]. Genomic DNA was digested with the restriction enzymes *Eco*RI and *Mse*I. Pre-amplification was carried out using primers containing a single selective nucleotide (A). The second amplification step was performed with a radioactively labelled *Eco*RI primer carrying two selective nucleotides (AC) in combination with either one of 4 *Mse*I primers containing three selective nucleotides (AAC, ACC, ACT, ATT). All primers were obtained from Genset (Paris, France). AFLP products were separated electrophoretically on denaturing 4.5% polyacrylamide gels and visualised via conventional autoradiography. Amplification product profiles were scored for the presence or absence of bands.

Table 2. Summary of allele segregation in tetraploid hybrids and parental genotypes (VNTR cf. AFLP).

	VNTR		AFLP	
	n	%	*n*	%
Assays prescreened on parental genotypes	79		0	
Assays detecting reproducible profiles	48	61	4	100
Assays detecting parental polymorphisms	34	43	4	100
Reproducible alleles scored	86		140	
Mean number of alleles per assay	2.5		15.5	
Polymorphic alleles (parents)	61	71	52	37
Polymorphic alleles (parents and progeny)	76	88	62	44
Maternal alleles	28	37	31	52
Maternal alleles segregating in progeny	23	82	19	61
Maternal alleles solely segregating in 548-9	9	32	0	0
Paternal alleles	29	38	21	34
Non-parental alleles segregating in progeny	15	20	10	16

Data analysis

The data sets obtained from RAPD, VNTR and AFLP analyses were each subjected to similarity analysis based on Jaccard's index [12]. Pairwise comparisons based on the similarity matrix generated by this analysis were used to generate dendrograms of genetic relatedness (not shown). All analyses were carried out with the aid of a program run within the Genstat software [33]. Comparison of estimates of genetic similarity generated by different assays applied to the same population was carried out by calculating Spearman's rank coefficient of correlation [66].

Results

Genetic analysis of parental genotypes

A total of 79 primer pairs (hereafter referred to as primers) for specific microsatellite loci were used to screen the parental genotypes [Obino l'Ewai, (AAB) and Calcutta 4 (AA)]. Of these primers, 48 generated amplification products, and 34 detected polymorphisms between the two genotypes (Table 2). These 34 primers detected a total of 86 alleles of which 61 (71%) were polymorphic between the parental genotypes. The proportion of successful amplifications which detected polymorphisms was high (71%) with an average multiplex ratio (average number of alleles detected per assay) of 2.5. However, the proportion of primers failing to generate an amplification product was also high (39%).

A total of 38 RAPD primers were used to prescreen the parental genotypes from which a total of 251 reproducible alleles were scored (Table 3). In contrast to microsatellite marker analysis, a very high proportion of the total number of RAPD assays successfully detected polymorphisms between the parental genotypes. The proportion of alleles which were polymorphic between the parental genotypes was similar for both RAPD and VNTR. However, RAPD analysis detected a greater total number of polymorphisms (Table 3). This is in accordance with a previous observation that RAPD analysis resulted in higher estimates of interspecific diversity in soybean germplasm than estimates based on other assays [37], although this may be dependent on the plant material under study.

No prescreening of AFLP primers was conducted, yet all four enzyme-primer combinations detected polymorphisms. A total of 140 reproducible, easily scored amplification products were generated across the four reactions of which 62 were polymorphic amongst the parental and progeny genotypes (Table 2). This high multiplex ratio is consistent with previously published studies [56].

Allelic segregation in tetraploid and triploid progeny

A high proportion of microsatellite alleles were polymorphic within the progeny population, in comparison with AFLP alleles. However, 30 microsatellite assays were required to detect a similar total number of polymorphic alleles to that revealed by four AFLP

Table 3. Summary of allele segregation in putative triploid hybrids and parental genotypes (VNTR cf. RAPD).

	VNTR		RAPD	
	n	%	*n*	%
Assays prescreened on parental genotypes	79		38	
Assays generating reproducible profiles	48	61	35	92
Assays detecting parental polymorphisms	34	43	35	92
Reproducible alleles scored	86		251	
Mean number of alleles per assay	1.8		7.2	
Polymorphic alleles (parents)	61	71	154	61
Mean number of polymorphic alleles per assay	1.3		4.4	
Number of assays used to screen progeny	18		20	
Reliable alleles scored (parents and progeny)	58		139	
Polymorphic alleles (parents)	43	74	99	71
Non-parental alleles segregating in progeny	1	2	6	4
Monomorphic alleles (parents and progeny)	14	24	27	19

assays (Table 2). Of the microsatellite alleles donated by the maternal plantain parent (Obino l'Ewai) 82% were polymorphic between the hybrids (Table 2). Whereas, only 45% of the AFLP alleles donated by Obino l'Ewai were polymorphic within this population. However, 32% of the VNTR were due solely to a polymorphism in TMPx 548-9 while AFLP analysis detected only one polymorphism unique to this hybrid. When this hybrid is not considered in the comparison, the estimates of recombination based on the two assays are in much closer agreement, at around 50%. The detection of a high rate of recombination of maternal alleles by the two assays provides further support for the theory of recombination during the formation of $2n$ ($3x$) megaspores by triploid plantain [4].

A similar proportion of alleles donated by both Obino l'Ewai and Calcutta 4 were observed to segregate in the backcross progeny. This trend was reflected in both RAPD and microsatellite marker analyses (57% and 42%, respectively, of polymorphic alleles being donated by Obino l'Ewai). In view of the allopolyploid nature of tetraploid *Musa* hybrids, this suggests that there is no restriction of recombination during normal n ($2x$) gamete formation by the maternal genotype (TMPx 4698-1).

VNTR and AFLP analyses detected a relatively high proportion of non-parental alleles which segregated in the tetraploid progeny population (Table 2). For microsatellite analysis it has been suggested that such alleles could be the consequence of heteroduplex formation [4]. However, the detection of a similar frequency of such alleles by AFLP analysis may suggest a phenomenon inherent to the populations being studied. The presence of progeny bands absent in the parental genotypes is frequently observed in yeast where it has been attributed to transposon activity [2]. Likewise, a similar frequency of non-parental bands observed during analysis of VNTR in soybean populations was attributed to mutations at the microsatellite loci [7] while non-parental bands generated by RAPD analysis of rice populations has been attributed to somaclonal variation [9]. Both somatic [51] and somaclonal [59] variation appear to be common phenomena in *Musa* and although the underlying mechanisms have yet to be defined this may offer an explanation for these unexpected segregation patterns. The frequency of polymorphic non-parental alleles revealed by both VNTR and RAPD analyses was considerably lower in the triploid population as compared to VNTR and AFLP analyses of the tetraploid hybrids. This suggests a disparity in meiotic behaviour or post-hybridization stability between the two stages of the pedigree under investigation in this study (Figure 1).

Genetic similarity between progeny and parental genotypes

Tetraploid hybrids

The range of genetic similarity estimates from pairwise comparisons between hybrids and their parental genotypes was generally wider for VNTR analyses

Table 4. Percentage and rank similarity of tetraploid hybrids to their parental genotypes (Obino l'Ewai, OL; Calcutta 4, C4) based on VNTR analysis and AFLP analysis.

Hybrid	Hybrid similarity to OL				Hybrid similarity to C4			
	VNTR		AFLP		VNTR		AFLP	
	%	rank	%	rank	%	rank	%	rank
TMPx 548-4	56.2	4	51.5	11	30.5	11	29.4	1
TMPx 548-9	25.5	14	67.6	1	13.8	14	12.8	12
TMPx 2637-49	60.0	2	52.6	8	25.4	13	11.4	13
TMPx 4698-1	52.9	12	60.0	2	35.6	3	17.0	9
TMPx 4744-1	59.2	3	56.1	6	33.3	6	22.2	6
TMPx 5511-2	56.0	5	54.5	7	33.3	6	11.3	14
TMPx 5706-1	54.0	10	59.5	3	36.2	2	16.3	10
Hybrid 5860-1	45.8	13	52.4	9	30.9	10	25.0	3
TMPx 6930-1	55.1	8	51.2	12	32.2	8	23.3	5
TMPx 7002-1	61.7	1	51.1	13	30.0	12	29.2	2
TMPx 7356-2	55.1	7	56.4	4	34.5	4	18.2	8
Hybrid 11669-1	55.0	9	47.7	14	33.9	5	24.4	4
Hybrid 14563-1	53.2	11	52.4	9	38.9	1	22.2	6
Hybrid 15063-1	55.3	6	56.1	5	31.0	9	14.6	11
Rank correlation[1]	−0.39				−0.04			

[1]Spearman's rank coefficient of correlation.

than AFLP analyses (Table 4). Pairwise comparisons between hybrids based on VNTR analysis also resulted in a wider range (25–85%) than when based on AFLP analysis (45–75%). However, this was principally due to the high estimate of genetic divergence between TMPx 548-9 and all other hybrids based on VNTR analysis, which was influenced by the large number of non-parental alleles present only in this hybrid. Although AFLP analysis detected a similar overall number of non-parental bands in the progeny population, only in one instance was such an allele unique to TMPx 548-9. This suggests that although TMPx 548-9 exhibits a diverse pattern of microsatellite repeat lengths, this is not reflected in a similar level of diversity in its other genomic regions (targeted by AFLP analysis).

The mean similarity between hybrids (other than TMPx548-9) and Obino l'Ewai is 55.0% based on VNTR and 54.0% based on AFLP (not significantly different, $P > 0.05$). With the exception of TMPx548-9, AFLP analysis tended to estimate greater divergence of hybrids from their paternal genotype (Calcutta 4) than microsatellite marker analysis (Table 4). Estimates of similarity between hybrids (other than TMPx 548-9) and Calcutta 4 varied considerably between the different types of analysis, with means of 32.7% and 20.3% for VNTR and AFLP, respectively ($P < 0.001$). Mean pairwise similarity estimates between hybrids (excluding TMPx548-9) were 67.3% for VNTR and 59.1% for AFLP ($P < 0.01$). This is in contrast to previous reports in which microsatellite marker analysis tended to detect polymorphisms to a greater extent than did AFLP analysis [37]. However, this may be a consequence of the interspecific pedigree of this hybrid population.

Some pairwise groupings were maintained between VNTR and AFLP analyses, such as TMPx 5511-2 and hybrid 15063-1, TMPx 548-4 and TMPx 4744-1, and TMPx 5860-1 and TMPx 6930-1 (data not shown). However, the rankings of hybrid similarity to Obino l'Ewai based on VNTR and AFLP analyses were not significantly correlated [66]. Similarly, the rankings of hybrid similarity to Calcutta 4 were not significantly correlated (Table 4). Thus, the overall genetic relationship between individual tetraploid hybrids and their parental genotypes based on VNTR analysis appeared quite different from that based on AFLP analysis.

Triploid hybrids

Genetic similarity estimates from pairwise comparisons between hybrids and their parental genotypes covered a wider range when based on VNTR analysis

than that based on RAPD analysis (Table 5). A similar trend was observed for pairwise comparisons between hybrids based on VNTR (10–75%) and RAPD (25–70%) analyses.

All hybrids were more dissimilar to the maternal grandparental genotype (Obino l'Ewai) than to TMPx 4698-1, as was expected of progeny from a backcross between TMPx 4698-1 and its paternal genotype (Calcutta 4) (data not shown). However, the mean similarity of hybrids to Calcutta 4 (38.8% and 38.3% for VNTR and RAPD, respectively) was considerable lower than would be expected from a normal inheritance model. Both VNTR and RAPD analyses identified two hybrids which were more dissimilar to Calcutta 4 than TMPx 4698-1. The relatively low similarity of the resultant backcross hybrids to Calcutta 4 may be a consequence of inheriting intact B genome chromosomes.

The rankings of hybrid similarity to Obino l'Ewai based on VNTR and RAPD analyses were not significantly correlated [66] although the rankings of hybrid similarity to Calcutta 4 were significantly correlated ($P < 0.05$, Table 5). Some pairwise groupings were maintained between the two assays, such as BC4 and BC14, BC23 and BC28, BC10 and BC13, BC12 and BC19, and BC16 and BC20 (data not shown). However, in general the genetic relationship between individual hybrids and their parental genotypes based on VNTR analysis appears quite different from that based on RAPD analysis.

Discussion

Implications for molecular breeding of plantain

Initial plantain breeding strategies at IITA required a very large number of crosses to be made in order to generate a very small number of tetraploid hybrids, most of which exhibited good agronomic characteristics [60]. In contrast, current efforts in plantain breeding at IITA are focused on the generation of secondary triploid *Musa* hybrids from crosses between tetraploid hybrids and diploid accessions [30]. This breeding scheme generates a very large number of hybrids, a high proportion of which have very poor agronomic performance. Consequently, marker-assisted indirect selection carried out at the nursery stage may be an important means of alleviating the need to establish vast numbers of hybrids in early evaluation field trials. It may also be possible to apply fingerprinting of potential parental genotypes in order to reduce the number of progeny populations that need to be generated in the first instance.

There has been much discussion in the literature concerning the reliability and transferability of RAPD amplifications [10, 35, 16, 49]. Within a single lab. operation we have found, in common with others [24, 34, 50, 40], that reliable RAPD data can be generated through precise standardization of appropriate protocols, replication of ambiguous assays and stringent interpretation of results. However, in practical terms, such procedures appear most inefficient in comparison to microsatellite marker analysis, when appropriate primers are readily available.

A large proportion (39%) of microsatellite markers used in this study failed to generate an amplification product. The absence of amplification products may be due to the loss of, or mutation in, regions homologous to the primer sequences. However, it is more likely that the absence of amplification products is due to the selection/synthesis of ineffective primers. The process of redesigning and synthesizing primers is time consuming and expensive. However, once suitable primers have been identified, they are easily available for general use by the scientific community. In the case of *Musa*, several hundred microsatellite markers have now been generated in advanced labs. across the world, particularly in the USA [14] and France [17]. For this reason, practical issues concerning the generation of microsatellite markers are no longer considered a rate limiting factor.

Microsatellite markers are a reliable means of detecting high levels of polymorphism using relatively simple techniques. In this study we have applied agarose gel electrophoresis for the visualization of VNTR, as this is the most appropriate technology for routine large scale DNA marker analysis in tropical breeding stations. However, many workers prefer the use of polyacrylamide gel electrophoresis using denatured gels and radioactive labelling, or automated analysis. Clearly, such approaches are likely to facilitate the identification of even greater levels of detectable polymorphism [34].

The codominant nature of microsatellite markers is a major theoretical advantage of VNTR compared to RAPD analysis. However, *Musa* germplasm, at all ploidy levels, appears to have a complex genomic nature. Thus, diploid, triploid and tetraploid *Musa* hybrids derived from an allopolyploid parental genotype may possess both A and B genome sequences, and intragenomic duplications. This genomic complexity may result in the loss of the co-dominant nature of

Table 5. Percentage and rank similarity of putative triploid hybrids to their parental genotypes (Obino l'Ewai, OL; Calcutta 4, C4) based on VNTR analysis and RAPD analysis.

Hybrid	Hybrid similarity to OL				Hybrid similarity to C4			
	VNTR		RAPD		VNTR		RAPD	
	%	rank	%	rank	%	rank	%	rank
BC1	36.4	12	27.3	25	40.5	12	38.6	16
BC2	42.4	6	33.3	16	38.5	15	42.9	12
BC3	40.6	9	35.1	14	36.8	16	40.4	15
BC4	32.4	18	25.8	26	44.4	6	51.1	2
BC5	32.4	18	32.3	20	44.4	6	46.9	5
BC6	26.1	26	42.2	10	24.1	27	44.6	10
BC7	36.4	12	29.8	23	40.5	12	45.7	7
BC8	41.9	7	24.6	28	34.2	23	41.9	13
BC9	31.4	20	32.8	18	47.2	5	44.9	8
BC10	27.0	24	32.3	20	54.3	2	46.9	5
BC11	41.2	8	41.1	11	41.0	11	32.7	20
BC12	35.5	14	45.8	7	36.1	18	35.7	18
BC13	28.6	22	32.8	18	48.6	4	44.9	8
BC14	21.6	27	25.4	27	57.6	1	53.3	1
BC15	33.3	15	51.0	5	34.3	22	21.8	26
BC16	43.7	5	33.3	16	35.9	19	37.5	17
BC17	48.3	2	40.4	12	28.2	25	26.0	25
BC18	38.2	10	53.1	3	42.1	10	18.2	27
BC19	35.3	15	47.4	6	43.2	8	32.1	21
BC20	26.7	25	30.2	22	36.4	17	50.0	3
BC21	37.0	11	39.3	13	25.7	26	41.5	14
BC22	30.0	21	51.8	4	35.3	20	29.3	24
BC23	35.3	15	42.9	9	43.2	8	32.1	21
BC24	17.2	28	45.5	8	39.3	14	35.2	19
BC25	62.5	1	58.3	1	15.8	28	15.8	28
BC26	46.7	3	55.2	2	32.4	24	31.1	23
BC27	45.2	4	27.9	24	35.1	21	47.8	4
BC28	28.1	23	34.4	15	50.0	3	44.0	11
Rank correlation[1]	0.20				0.47*			

[1]Spearman's rank coefficient of correlation
* $P < 0.05$.

many microsatellite markers, due to the difficulties associated with designating allelic relationships. Although Southern hybridizations may eliminate some undefined amplification products, this technique is not appropriate for the high throughput analysis required in a molecular breeding program. The loss of co-dominant marker information also has important implications for estimating genetic relationships between polyploid genotypes. For example, if one locus has different alleles on both A and B genome chromosomes then these are likely to be scored as different loci thereby exaggerating the estimate of genetic diversity. Nevertheless, microsatellite markers remain more informative than RAPD assays due to their association with known genomic sequences and their more reliable amplification via stringent PCR conditions.

In this study we have also shown that, contrary to previous reports in other systems [37], the level of AFLP polymorphisms in *Musa* is comparable to that generated by microsatellite marker analysis. This is based on the use of ethidium stained agarose gel electrophoresis of VNTR compared with polyacrylamide gel separation and autoradiography detection of AFLP. Similarly, both RAPD and VNTR analyses proved effective, in general, for estimating genetic diversity. However, this type of analysis is more ef-

ficiently accomplished using AFLP assays. Similarly, AFLP analysis would appear to be the most effective means of fingerprinting parental genotypes for genetic diversity analysis.

The potential co-dominant nature of microsatellite markers and the ease with which microsatellite markers can be multiplexed [26, 55] maintain a substantial comparative advantage for the application of this technique in marker assisted selection programs. In such applications a large number of genotypes need to be screened with a relatively small number of markers, preferably within the breeding station. Marker-assisted backcross breeding, to eliminate individuals with a large proportion of the exotic parent's genome, presents a similar logistical scenario.

Considerable advances have recently been made in the development of techniques for the routine conversion of AFLP markers into simple PCR assays [1, 11, 46]. If this strategy proves effective in complex polyploids such as *Musa*, this may warrant the use of AFLP technology in an advanced lab. for the rapid and efficient identification of markers for important agronomic characters. A specific marker may then be converted into a simple PCR assay for easy routine screening within the breeding station.

In this study, the genetic relationship between individual *Musa* hybrids and their parental genotypes based on VNTR analysis appeared quite different from that based on AFLP or RAPD analysis. This poor correlation between estimates of genetic similarity based on RAPD, VNTR and AFLP, suggests that the different PCR-based assays may selectively screen complementary, rather than overlapping, regions of the *Musa* genome. This is possible as microsatellite markers target a specific class of sequences which are likely to have specific behaviour in terms of mutation rate and duplication rate etc. In contrast, it is expected that AFLP analysis should randomly screen the entire germplasm. However, in practice it is difficult to identify a restriction enzyme which recognizes sequences that are evenly distributed across the genome. This disparity between assays may provide a partial explanation for the failure of microsatellite marker analysis to predict the breeding value of parental genotypes [52]. Thus, it may be necessary to utilize a range of marker systems in order to generate highly accurate estimates of genetic similarity in germplasm analysis and parental fingerprinting studies.

Development of Musa *breeding strategies based on PCR-based marker analysis*

In this report we have presented AFLP and additional microsatellite marker data in support of the theory of recombination during the formation of $2n$ ($3x$) megaspores by triploid plantain [4]. This implies that, contrary to the commonly accepted premise (reviewed in [44, 63]), the triploid *Musa* genome is not fixed and the breeding of polyploid *Musa* crops is not restricted to the development of improved diploid hybrids. Thus, recombination can be effectively utilized from both parental genotypes in order to improve this crop through traditional cross-breeding strategies. This is particularly important in terms of taking advantage of beneficial epistasis resulting from the recombination of linked loci in both parents [41, 28].

Analysis of progeny populations from a backcross of a tetraploid hybrid to its diploid parental genotype suggest that, notwithstanding the problems of inbreeding depression, this is a highly ineffective means of accumulating the genetic background of the diploid accession in triploid hybrids.

Data presented in this study suggest that there is no restriction of recombination during normal n ($2x$) gamete formation by the tetraploid maternal genotype (TMPx 4698-1). Hybrid TMPx 4698-1 has been shown to have an AAAB genomic constitution [31]. On this basis, there would appear to be considerable tolerance of homoeologous chromosome pairing by tetraploid *Musa* hybrids which is probably facilitated by the low level of differentiation between A and B genomes [31]. The genetic control of homologous chromosome pairing, and thereby the restriction of homoeologous recombination, has been intensively studied in wheat [15, 19] where its manipulation remains the most efficient means for introgressing genes from distant germplasm [22, 38]. Homoeologous recombination has also been reported in other crops, such as tomato [32], potato [64] and oilseed rape [48]. Although specific regulatory loci have not been identified in these crops, it is clear that the control of homologous recombination breaks down during interspecific and/or interploidy hybridizations. For all these crops, including *Musa*, high levels of homoeologous recombination can provide a useful means to introgress characters from exotic germplasm.

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